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**The Effect of Prolactin on the Number of Membrane-Associated Particles in Kidney Cells of the Euryhaline Teleost *Gasterosteus aculeatus* during Transfer from Seawater to Freshwater**

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THE EFFECT OF PROLACTIN ON THE NUMBER  
OF MEMBRANE-ASSOCIATED PARTICLES IN  
KIDNEY CELLS OF THE EURYHALINE  
TELEOST *GASTEROSTEUS ACULEATUS*  
DURING TRANSFER FROM SEAWATER  
TO FRESHWATER: A FREEZE-ETCH STUDY

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SUMMARY

The membranes of kidney cells of 3-spined sticklebacks were examined in freeze-etch replicas. The numbers of particles adhering to surfaces and fracture faces of the outer cell membranes and the membranes of the basal labyrinth were determined. The latter membranes probably are the main location of ion-transporting enzyme complexes.

The total number of particles per cell in freshwater fish exceeds that of seawater fish by about 50 % for the outer cell membrane, and by almost 200 % for the membranes of the basal labyrinth.

After transfer of seawater fish to freshwater, particle numbers increase and their densities approximate freshwater values after 20 h. This rise in particle numbers coincides with the increase of ion-transporting activity of the cells known to take place after transfer to freshwater.

The rate of increase of particle densities is enhanced after injection of ovine prolactin. This hormone is known to stimulate  $\text{Na}^+/\text{K}^+$ -ATPase activity of the basal labyrinth of teleost kidney cells.

The results indicate that the particles represent enzyme complexes. The number of particles is probably under hormonal control. The increase in particle densities after transfer to freshwater is accompanied by a rise in the number of nuclear pores, which is noticeable by 10 h.

No changes were observed in the density of the particles adhering to the fracture faces of gap junctions.

INTRODUCTION

Replicas of freeze-fractured and etched cells offer the opportunity to study cell membranes at high resolution. Most types of membranes studied in this way have in common that the surfaces and fracture faces are covered by varying numbers of small particles with a diameter between 6 and 12 nm. It has been suggested by several authors (Deamer & Branton, 1967; Bretscher, 1973) that such particles represent proteins or lipoprotein units accounting for the physiological functions of the membranes concerned. In a former study of kidney cells of the 3-spined stickleback

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*Gasterosteus aculeatus* (Wendelaar Bonga & Veenhuis, 1974), particle numbers were determined for 2 types of membranes, the outer cell membranes and the membranes forming the basal labyrinth. It was shown that the density of the membrane-associated particles, in freshwater fish, is specific and remarkably constant for each of the 2 membrane types studied. In this paper, dealing with the same cell type, we present evidence that changes induced in membrane function are correlated with changes occurring in the numbers of particles per unit area. It is furthermore shown that particle numbers are changed by a hormone known to alter the function of the membranes concerned.

The cells studied, those forming the epithelium of the second proximal tubules, are involved in ion transport activity. The enzyme complexes accounting for ion transport are presumed to be membrane-bound and to be located mainly in the membranes forming the basal labyrinth, as is common for kidney cells in general (Ericsson & Trump, 1969). The sticklebacks used, those of the form *trachurus*, migrate in Spring from the sea to freshwater and return to the sea in late summer, after the reproductive period. Consequently, the kidney cells undergo marked changes in ion-transporting activity twice a year. The main osmoregulatory adjustments occurring in kidney cells of migrating teleosts are well known (Hickman, 1968; Hickman & Trump, 1969; Miles, 1971). In a marine environment, there is mainly a modest secretion of divalent ions like  $Mg^{2+}$  and  $SO_4^{2-}$ . In freshwater, an intense reabsorption of monovalent ions,  $Na^+$  and  $Cl^-$ , is dominant (Hickman & Trump, 1969). The occurrence of this dramatic change in ion-transport activity makes the kidney cells of the stickleback a favourable object in which to study the relationship between functional changes and change in the numbers of membrane-associated particles. The results of density counts on the outer cell membranes and the membranes of the basal labyrinth are presented. A comparison is made between freshwater and seawater sticklebacks. In addition, kidney cells of seawater sticklebacks were examined after exposure to fresh water for 10 and 20 h.

The osmoregulatory adjustments of seawater fish entering freshwater are known to be accelerated by prolactin (Lam & Hoar, 1967; Lam & Leatherland, 1969). Ovine prolactin was therefore injected at the moment of transfer, in order to establish whether enhancement of the rate of physiological adaptation also leads to an increased rate of change in particle numbers.

Nuclear pores and gap junctions were also studied. Freeze-etch replicas are favourable for the observation of nuclear pores, since large areas of the nuclear envelopes are frequently exposed. Density counts were made in order to determine whether the number of pores is useful as a parameter of cellular activation in short-term experiments.

Gap junctions are intercellular connexions which are presumed to be places of ionic and metabolic coupling of adjacent cells (Gilula, Reeves & Steinbach, 1972). Observations were therefore made to determine whether or not changes in cellular activity are reflected in the structure of these junctions.

## MATERIALS AND METHODS

The sticklebacks used were adult female specimens of the *trachurus* form, with a body length varying between 65 and 70 mm. Freshwater fish were obtained from laboratory stock. Seawater specimens were caught in the Wadden Sea, in winter. The fishes were acclimatized for at least 3 weeks at a temperature of about 15 °C with a daily light period of 8 h. They were kept in containers with tap water or natural seawater and killed by decapitation.

For the transfer experiments, groups of sticklebacks were transferred directly from seawater to freshwater, 8 h after the start of the dark period. In one group, ovine prolactin was injected intraperitoneally at the moment of transfer. Every specimen received a single injection of 0.4 i.u./g. Each dose was dissolved in 0.05 ml of 0.6 % NaCl solution in distilled water. Controls were injected with the same volume of solvent. The hormone was kindly provided by the Hormone Division of the National Institutes of Health, Bethesda, Md. (N.I.H.-P-Sg; mean potency 30.30 i.u./mg; growth hormone activity less than 0.010 USP units/mg).

Freeze-etch replicas were made of small tissue blocks, immersed in 25 % glycerol in 0.1 M phosphate buffer (pH 7.2) for 15 min. The tissue blocks were frozen in liquid Freon for 1 s, and stored in liquid nitrogen. Replicas were made using Balzers BA360M Freeze Etch equipment. Fractured specimens were etched, and shadowed with platinum and carbon. The replicas were cleaned in a saturated solution of  $K_2Cr_2O_7$  in 70 %  $H_2SO_4$  for some hours, and subsequently in 40 % NaOH solution for 1 h. After rinsing in distilled water the replicas were examined in a Philips EM 300 electron microscope.

The results were analysed statistically by Student's *t*-test.

## OBSERVATIONS

The cells forming the epithelium of the second proximal tubules are prismatic, with an apical brush border. The central and basal parts of the cytoplasm contain the basal labyrinth. In sticklebacks, this labyrinth differs structurally from comparable membrane systems in kidney cells of mammals, such as the rat. In the latter animal the labyrinth is formed by the outer cell membranes, which line interdigitating cytoplasmic processes (Bulger, 1965). In sticklebacks, the labyrinth is not formed by the outer cell membrane. It consists of intracellular membranes lining branched and blindly ending narrow saccular spaces. These spaces communicate with the extracellular space by rows of small pores, located in the lateral and basal parts of the outer cell membrane. A detailed description of these cells, based on thin sections and freeze-etch replicas, has been presented before (Wendelaar Bonga & Veenhuis, 1974).

The number of particles per unit surface of membrane ( $1 \mu m^2$ ) was determined for the surfaces and fracture faces of the outer cell membranes and the membranes of the basal labyrinth. According to Branton (1966) freeze-fracturing and etching of a membrane leads to exposure of 4 possible faces: an inner surface, in contact with the cytoplasm (IS), an outer surface (OS), and two faces resulting from splitting of the membrane along its central, hydrophobic, plane. Splitting results in exposure of one out of two fracture faces, one originally oriented to the interior (the outer fracture face, OFF) and the other facing the exterior (the inner fracture face, IFF) of the cell or organelle (see fig. 1 in Wendelaar Bonga & Veenhuis, 1974). The terminology was adopted from Kopp (1972) and Mühlethaler (1972). The relationship to another labelling convention (McNutt & Weinstein, 1970) is as follows: IS:C; IFF:A; OFF:B; OS:D. All 4 possible faces of both membrane types are present in the replicas of the kidney cells, albeit in different frequencies. For unknown reasons, the

IS and the IFF are most common (Figs. 4, 5), whereas the OS, and especially the OFF are rarely found. This implies that many replicas are needed in order to obtain sampling areas of the required size. For the groups of fish from freshwater and from the sea such samples were obtained for all 4 faces of both membrane types. For the other, experimentally treated, groups the numbers per  $\mu\text{m}^2$  of OFF were extrapolated from smaller areas ( $0.2\text{--}0.5 \mu\text{m}^2$  per animal).

The pores in the nuclear envelope are clearly revealed in the replicas (Fig. 4). The number of pores was determined per unit of  $25 \mu\text{m}^2$  of membrane surface. Only rather flat areas of the nuclear membranes were selected as sampling areas, in order to reduce deviations due to curvature of the membranes.

The density of the particles occurring in gap junctions was also determined in the experimental groups. Gap junctions are plaque-like intercellular junctions. They occur distributed over the apical parts of the lateral cell membranes. In osmium-fixed tissue sections the opposing cell membranes run closely parallel in the junctional area, separated by an electron-transparent gap about 2 nm in width. In freeze-etch replicas, the most prominent structural feature of the junctions is the presence, on the IFF, of densely packed particles with a characteristic diameter of 9 nm (Fig. 6). Such particles are characteristic of the IFF (EMF, Goodenough & Revel, 1970; face A, Raviola & Gilula, 1973) of vertebrate gap junctions. The corresponding area of the IS was identified as a slightly depressed, rather smooth plaque. This identity was inferred from the frequent observation of junctional membranes which are only partly split, revealing the IS of one membrane and the IFF of the opposing membrane (Fig. 7). The pictures suggest that the particles are projecting from the IFF of the junction and penetrate into the opposing membrane, where they are also connected with the IFF. This interpretation is tentative, however, since the OS and OFF of the gap junctions were not found.

For an evaluation of the results of the counts of particles and nuclear pores, not only their density but also their total number per cell has to be taken into account. The height of the cells of the second proximal tubules is greater in freshwater fish than in seawater specimens, while the volume of the nuclei and the surface of the membranes of the basal labyrinth are also greater in freshwater fish (Wendelaar Bonga, 1973*a*). Thus, when seawater fish are transferred to freshwater, an increase may be expected of the surfaces of the outer cell membranes, the membranes of the basal labyrinth, and the nuclear envelope. Such increases have indeed been found. The parameters mentioned reached values characteristic for freshwater animals within 6–9 days after transfer to freshwater (Wendelaar Bonga, 1973*b*). In the first 3 days after transfer no changes were noticeable. Hence, for the short-term transfer experiments described in this study, growth phenomena have not been taken into consideration. On the other hand, for an interpretation of the results of the density counts of freshwater and seawater sticklebacks, the differences in extent of the membranes have to be taken into account. For this reason data on cell height, nuclear size and extent of the basal labyrinth are presented. Cell height and volume of the nuclei were determined in paraffin sections in the light microscope. The extent of the membranes of the basal labyrinth was estimated by stereometrical analysis of electron micro-

graphs of ultrathin sections. Details of the techniques used have been described before (Wendelaar Bonga, 1973*a*).

*Kidney cells of sticklebacks from the sea*

The animals used were caught at sea in December, i.e. several months after seaward migration. The quantitative data relating to cells of the second proximal tubules obtained from 5 animals are presented in Table 1 and, as far as the particles are concerned, in Fig. 1.

Table 1. *Morphometric data of the cells of the second proximal tubules of sticklebacks*

	Seawater	Freshwater
Cell height, $\mu\text{m}$	$8.8 \pm 0.5$	$9.4 \pm 0.6$
Cell width, $\mu\text{m}$	$3.0 \pm 0.1$	$3.2 \pm 0.1$
Membrane surface ( $\mu\text{m}^2$ ) of basal labyrinth/ $\mu\text{m}^3$ of cytoplasm	$3.5 \pm 0.5$	$6.3 \pm 1.1$
Membrane surface ( $\mu\text{m}^2$ ) of basal labyrinth per cell	$219 \pm 24$	$466 \pm 57$
Surface ( $\mu\text{m}^2$ ) of basal and lateral cell membrane per cell	$93 \pm 4$	$103 \pm 5$
Surface of nuclear envelope, $\mu\text{m}^2$	$51 \pm 2$	$56 \pm 2$
No. of nuclear pores/ $25 \mu\text{m}^2$	$146 \pm 12$	$180 \pm 11$
No. of particles/ $\mu\text{m}^2$ of the IFF of gap junctions	$7980 \pm 161$	$8048 \pm 187$

Mean values ( $\pm$  S.E.) are given for 2 groups of 5 specimens (one group from the sea, the other from freshwater).

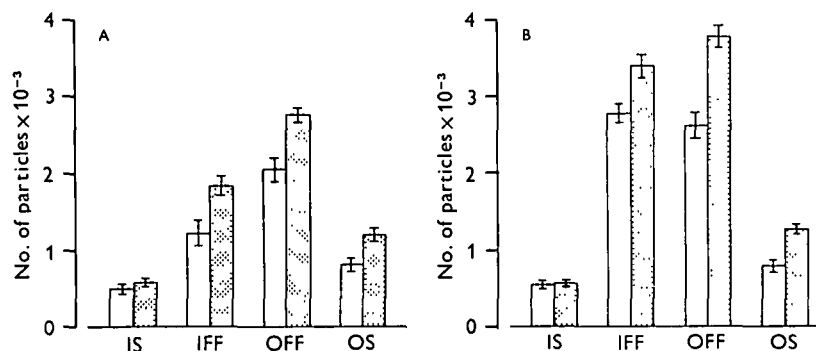


Fig. 1. The number of particles per  $\mu\text{m}^2$  of the surfaces and fracture faces of the outer cell membranes (A) and the membranes of the basal labyrinth (B). Means  $\pm$  S.E. are given for 2 groups of 5 sticklebacks, one group from seawater (white bars), the other from freshwater (mottled bars).

The standard errors of the means of the particle densities of both the cell membranes and the membranes of the basal labyrinth appear to be relatively small. They are all less than 10%. This indicates that particle densities are highly characteristic for each of the surfaces and fracture faces of these membranes. The densities found on the fracture faces surpass those of the surfaces, while the numbers present on the OS are higher than those on the IS. A comparison of the outer cell membranes with

the membranes of the labyrinth reveals that the densities of the surfaces are rather similar ( $P > 0.05$ ). The densities of the fracture faces of the basal labyrinth are considerably higher than those of the fracture faces of the cell membranes ( $P < 0.001$ ).

#### *Kidney cells of freshwater fishes*

As in seawater fishes, the particle densities of the fracture faces exceed those of the surfaces, while the OS shows higher numbers than the IS. This holds for both types of membrane (Fig. 1).

If the particle densities of the outer cell membranes are compared with those of the membranes of the basal labyrinth, differences are apparent as far as the fracture faces are concerned. The densities of the IFF and OFF of the basal labyrinth are markedly higher than those of the outer cell membranes ( $P < 0.001$ ).

A comparison of the densities of the freshwater and seawater groups shows clearly that the highest densities are found in the freshwater fishes. This holds for all faces of both types of membranes.

For the outer cell membranes the differences amount to 15 % for the IS ( $P \leq 0.05$ ), 50 % for the IFF ( $P < 0.001$ ), about 30 % for the OFF ( $P < 0.001$ ) and 45 % for the OS ( $P < 0.001$ ). The mean density of the IS of the basal labyrinth is only slightly higher (5 %) in freshwater ( $P > 0.05$ ), but the differences are highly significant ( $P < 0.001$ ) for the IFF (26 %), the OFF (44 %), and the OS (60 %).

The differences between seawater and freshwater fish are even more marked when the numbers of particles per cell are compared. The data on height and width of the cells and on the extent of the basal labyrinth (Table 1) show that the freshwater cells are larger than the seawater cells. A comparison of the dimensions of the cells reveals that in the freshwater fish the mean surface area per cell of the lateral and basal parts of the cell membranes is 10 % larger, and that of the membranes of the basal labyrinth is over 100 % larger, than in seawater fish. It can be calculated from these data that the number of particles per cell, present on the lateral and basal parts of the outer cell membrane of freshwater fish, exceeds that of seawater fish by about 50 %. For the basal labyrinth the difference is much greater. The total number per cell of the particles adhering to the membranes of the basal labyrinth is greater by almost 200 % in the freshwater fish.

The density of the nuclear pores of the freshwater group is more than 20 % higher in the freshwater group (Table 1). The surface of the nuclear envelope is also about 10 % larger. These differences imply that the total number of nuclear pores of a nucleus of a freshwater cell exceeds that of a seawater cell by almost 30 %.

#### *Cells of seawater fish exposed for 10 or 20 h to fresh water*

If in good condition, seawater sticklebacks survive immediate transfer to freshwater without noticeable harm. Mortality did not occur during our experiments. It is known that exposure to freshwater leads to rapid changes in the osmoregulation of seawater sticklebacks. Volume and composition of the urine are altered within a few hours after transfer from seawater to freshwater (Lam & Hoar, 1967). If membrane-bound

particles are units engaged in kidney function, it may be expected that particle densities of the fish increase shortly after transfer. This supposition is confirmed by the results of the short-term exposure experiments (Fig. 2). They show that the particle densities increase towards densities found in freshwater fish. Changes are evident already after 10 h. After that period, particle densities of most membrane faces are intermediate between the values found in the seawater fish and those of the freshwater groups. After 20 h, the densities of most faces approximate the freshwater values.

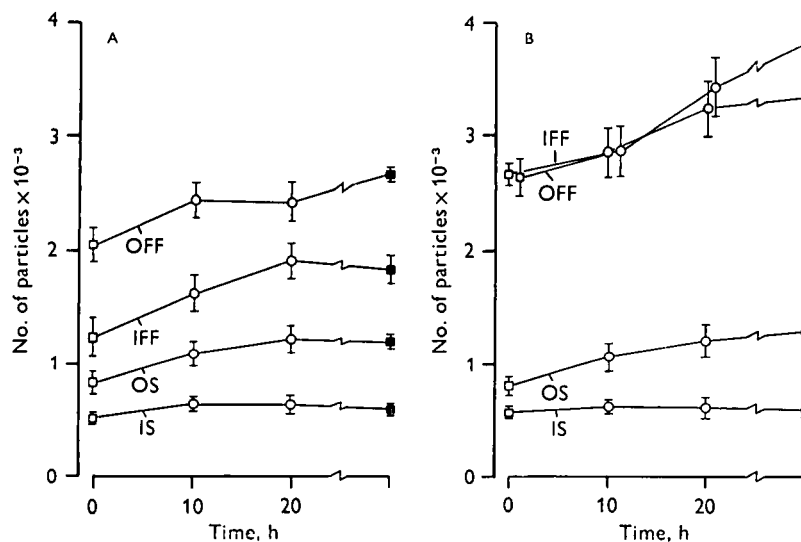


Fig. 2. The number of particles per  $\mu\text{m}^2$  of the surfaces and fracture faces of the outer cell membranes (A) and the membranes of the basal labyrinth (B). Means  $\pm$  S.E. are given for 2 groups of 3 seawater sticklebacks, one adapted for 10 h and the other for 20 h to freshwater. The data for the seawater (open squares) and freshwater (black squares) groups are given as reference values.

The density of the nuclear pores increased notably during the first 10 h, from  $146 \pm 12$  to  $173 \pm 15$  per  $25 \mu\text{m}^2$  ( $P < 0.05$ ). After 20 h, the density amounted to  $185 \pm 16$ , which is similar to the value for the freshwater group.

The number of particulate units of the IFF of the gap junctions did not change after transfer ( $7862 \pm 197/\mu\text{m}^2$  after 10 h;  $7910 \pm 261/\mu\text{m}^2$  after 20 h).

#### *Cells of prolactin-injected seawater fish exposed for 10 h to fresh water*

A group of 5 seawater sticklebacks received a single injection of ovine prolactin (0.4 i.u./g) at the moment of transfer to freshwater. A group of 5 similarly treated fish, which were injected with the solvent only, were used as controls. After an exposure period of 10 h the cells of the second proximal tubules were examined. The results of the particle counts (Fig. 3) reveal that the membranes of the prolactin-injected fish show higher mean densities than those of the controls. This holds for all faces,



with the exception of the IS of the outer cell membranes and the membranes of the basal labyrinth. The differences are statistically significant for the IFF ( $P < 0.001$ ) of both types of membranes, and for the OS ( $P < 0.01$ ) of the membranes of the basal labyrinth.

The number of nuclear pores ( $173 \pm 19/25 \mu\text{m}^2$ ) was similar to that of the controls ( $169 \pm 16/25 \mu\text{m}^2$ ). No differences were observed in the structure of the gap junctions.

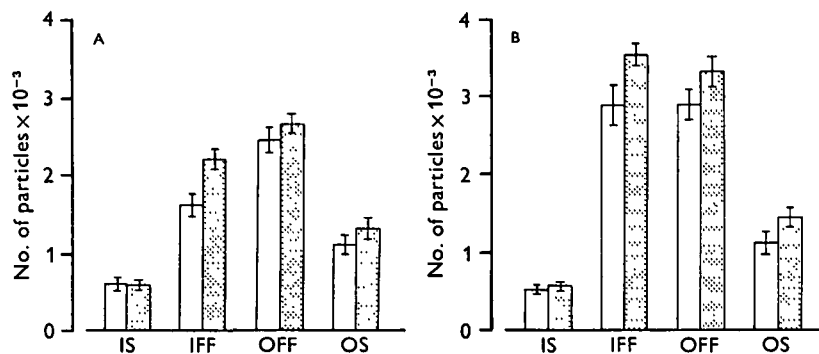


Fig. 3. The number of particles per  $\mu\text{m}^2$  of the surfaces and fracture faces of the outer cell membranes (A) and the membranes of the basal labyrinth (B). Means  $\pm$  S.E. are given for 2 groups of 5 seawater sticklebacks, exposed for 10 h to freshwater. Mottled bars, specimens injected with 0.4 i.u./g of ovine prolactin at the moment of transfer; white bars, solvent-injected controls.

#### DISCUSSION

Surfaces and fracture faces of membranes of plant and animal cells are covered by small particles, which are partly embedded in a smooth matrix. Since most particles are removed by protein-digesting enzymes (Branton, 1971), they probably represent protein or lipoprotein units. The membrane picture arising from freeze-etch studies is, therefore, consistent with biochemical and biophysical evidence that most or all membrane-bound proteins are particulate units, partly embedded in a lipid bilayer, as proposed in the fluid lipid/crystal protein model (Lenard & Singer, 1966; Singer & Nicolson, 1972).

It has been suggested that the density of the particles reflects the metabolic activity of the membranes concerned (Branton, 1969). Orci & Perrelett (1973) found, in smooth muscle cells of the mouse, that the number of membrane-bound particles is increased at sites of formation of pinocytotic vacuoles. Such data favour the idea that most membrane-bound particles are functional units, engaged in specific membrane functions (see also Bretscher, 1973). But attempts to correlate changes in membrane functions with differences in particle densities are scarce and not fully convincing so far. In erythrocyte membranes, Kirk & Tosteson (1973) found specific differences in particle densities of the IFF between various mammalian species. The authors found a positive correlation of these differences with known variations in the rates of active potassium transport. No changes, however, were found after treatment with valinomycin and amphotericin, antibiotics known to alter the ion-permeability characteristics of erythrocyte membranes. Tourtelotte & Zupnik (1973) have reported a

reduction of particle numbers in puromycin-treated membranes of *Acholeplasma laidlawii* cells. This decrease has been correlated with the inhibitory effect of this chemical on the uptake of glucose. Packer, Williams & Criddle (1973) have compared mitochondrial membranes from wild type and functionally deficient yeast strains. Loss of respiratory enzyme activity and of oligomycin-sensitive ATPase was not accompanied by notable changes in size or distribution of the membrane particles. As is stressed by these authors, however, loss of functional capacities in mutant strains does not exclude the possibility that the protein complexes accounting for the functions concerned are still present, albeit in an inactive state. This possibility is not unlikely, since Schatz *et al.* (1972) found that most of the apo-protein portion of cytochrome oxidase and of oligomycin-sensitive mitochondrial ATPase remain associated with the mitochondrial membranes, even when the enzymes are not normally functioning.

The above-mentioned experiments were aimed at correlating a reduction of the particle numbers with a reduced functional activity. The purpose of our transfer experiments was the induction of an increase of the particle density by activation of the membranes. This approach seems preferable as long as the effects of inactivation on the fate of enzyme complexes is uncertain.

Our results point to a positive correlation between particle numbers and enzymic activity. This conclusion is not only based on the results of the transfer experiments. It is also suggested by the differences in particle densities found between the outer cell membranes and the membranes of the basal labyrinth in the cells of all experimental groups, and by the differences found between the membranes of freshwater and seawater fishes.

The particle density of the basal labyrinth was higher than that of the outer cell membranes, in all cells studied. Higher functional activity is probably also connected with the membranes of the basal labyrinth. This is indicated by physiological and histochemical data on teleost kidney cells, showing that the membranes of the basal labyrinth are highly specialized for ion transport activity (Ericsson & Trump, 1969).

The comparison of membranes of seawater fish with those of freshwater specimens revealed that particle densities are higher in the latter group. The difference of particle numbers per cell was limited to about 50% for the outer cell membranes, but amounted to no less than 200% for the membranes of the basal labyrinth. A major difference in kidney function between freshwater and seawater fishes concerns ion transport activity. The transport rate increases dramatically when euryhaline fish migrate from the sea to freshwater. There is, furthermore, a change in the direction of transport and in the nature of the ions concerned. In the sea, the cells of the second proximal tubules of euryhaline fish are engaged in the secretion of modest amounts of divalent ions like  $Mg^{2+}$  and  $SO_4^{2-}$ , and the reabsorption of some monovalent ions, mainly  $Na^+$  and  $Cl^-$ . In freshwater, an intense reabsorption of the monovalent ions and a small uptake of  $Mg^{2+}$  are known to take place (Hickman, 1968; Hickman & Trump, 1969; Miles, 1971). When entering freshwater, synthesis of ion-transporting enzyme complexes occurs, which enables the fish to cope with the changed environmental conditions. One of the enzymes is the  $Na^+/K^+$ -activated ATPase, as was demonstrated in the killifish *Fundulus heteroclitus* (Pickford *et al.* 1970). This enzyme

is associated with the membranes of the basal labyrinth, as has been established histochemically and biochemically in kidney cells of several vertebrates (Ericsson & Trump, 1969; Kinne & Schmitz, 1971). Thus, the higher number of membrane-associated particles found in the membranes of the basal labyrinth of freshwater fishes is positively correlated with a higher membrane-bound functional activity.

The results of our transfer experiments provide further evidence that the particles represent functional units. Investigations of Lam & Hoar (1967) on sticklebacks have revealed that changes in kidney functions are noticeable within some hours after transfer of the fish from the sea to freshwater. When the experiments are performed in winter, transfer is initially accompanied by a rapid fall of the osmolality of the blood plasma, partly due to the production of large volumes of concentrated urine. After 4–8 h, an increase in plasma osmolality is found, while the osmolality of the urine is reduced. This decrease reflects an enhanced rate of reabsorption of ions by the kidney tubules (Lam & Hoar, 1969). We found a marked increase of membrane-associated particles 10 h after transfer to freshwater. Accordingly, an increase in ion-transport activity in the kidney cells is accompanied by an increase of the numbers of membrane-associated particles.

Lam & Hoar (1967) demonstrated in addition that injection of mammalian prolactin facilitates the adaptation of seawater sticklebacks to freshwater. Prolactin-treated fish are able to restore plasma osmolality levels earlier than solvent-injected controls. This effect of mammalian prolactins is well known for euryhaline teleosts (compare Ensor & Ball, 1972). Enzymes stimulated by prolactin are  $Mg^{2+}$ - and  $Na^+/K^+$ -activated ATPase, as was demonstrated for kidneys of *Fundulus heteroclitus* (Pickford *et al.* 1970). It is clear from our results that prolactin injection at the moment of transfer to freshwater accelerates the increase of the particle densities. This result, which is a clear demonstration of a hormonal effect on the membrane-associated particles, seems to us substantial evidence in favour of the supposed identity of the particles with enzyme complexes.

The presence of higher numbers of nuclear pores in freshwater fish, when compared to the seawater specimens, points to a higher transport rate in the first group of substances like ribosomal and messenger RNA from the nucleus to the cytoplasm (compare Scheer, 1973). A positive relationship between cellular activity and the number of nuclear pores has been established by Lott, Larsen & Whittington (1972) in germinating cotyledons of *Cucurbita maxima*, and by Scheer (1973) in growing oocytes of *Xenopus laevis*. Our transfer experiments show that the increase in number of pores from seawater to freshwater values is a rapid process. A significant rise was already noticeable 10 h after transfer, i.e. long before other indications of increased cellular activity which can be obtained with morphometric techniques, such as increase in cellular and nuclear volume, become evident (Wendelaar Bonga, 1973*b*). Hence, the density of the nuclear pores proves to be a useful parameter for the rapid detection of changes in cellular activity.

The gap junctions in the kidney cells of sticklebacks show the same structural characteristics as those described in a variety of other vertebrate cells (Goodenough & Revel, 1970; Raviola & Gilula, 1973). These junctions are supposed to be sites of ionic

coupling of cells and the transport routes for a number of metabolically significant substances (Payton, Bennett & Pappas, 1969; Gilula *et al.* 1972). They are also presumed to account for the synchronous differentiation and functioning of groups of cells (Barnett & Szabo, 1973). For this reason it may be expected that an increase of the activity in a synchronously working group of cells, like those of the kidney tubules, is reflected in the structure of the gap junctions. The results of our particle counts show that activation of the kidney cells, as a result of transfer of sticklebacks from seawater to freshwater, did not influence the density of the particles adhering to the IFF of the gap junctions. It is possible, however, that increased cell activity leads to enlargement of the junctional areas, and thus of the total number of junctional particles per cell.

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Figs. 4-7. Freeze-etch micrographs of cells of the second proximal tubules. The encircled arrows in the left upper corners indicate the direction of metal shadowing; *iff*, inner fracture face; *is*, inner surface; *os*, outer surface.

Fig. 4. Central and basal areas of some cells from freshwater animal. The nucleus shows the inner nuclear membrane (*innm*), parts of the outer nuclear membrane (*onm*) and many nuclear pores (*p*). Membrane pairs (*mp*) forming the basal labyrinth are in contact with the basal cell membrane (arrows); *bla*, basal lamina; *gz*, Golgi zone; *lm*, lateral cell membrane; *mi*, mitochondria.  $\times 19200$ .



Fig. 5. Seawater animal. Outer cell membranes (*ocm*) and membranes of the basal labyrinth (*bl*); *mi*, mitochondrial membrane.  $\times 33\,500$ .

Fig. 6. Seawater animal. Apical area of the lateral cell membrane. The densely packed granules are characteristic for the inner fracture face of a gap junction (*gj*). The inner surface is shown of part of the lateral membrane of an adjacent cell; *tj*, tight junction.  $\times 36\,700$ .

Fig. 7. Seawater animal. Lateral cell membranes of 2 adjacent cells, connected by a gap junction (*gj*) which is partly split. The white rim marks the transition of the plane of fracturing from the inner surface of one cell to the inner fracture face of the adjacent cell. The inner surface of the junction is smooth and slightly depressed.  $\times 40\,500$ .

